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HISTOPATHOLOGICAL EFFECT OF *Giardia duodenalis* ON LIVER AND INTESTINAL TISSUES IN A BALB/C MOUSE MODEL

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ABSTRACT

In this study, we build upon previous research by investigating the histopathological effects of **Giardia duodenalis** isolated from humans and dogs using a mouse model. A total of 20 BALB/c mice (average age: 8 weeks $\pm$ 2.4 days; average weight: 19.8 $\pm$ 0.5 g) were randomly divided into two groups of ten mice each. The unchallenged control group received no challenges and was orally administered 0.3 mL of normal saline. In contrast, the group challenged with *Giardia duodenalis* received a solution containing **G. duodenalis** at a concentration of 2×10^4 cysts/mL in 0.3 mL, also orally. Clinical signs were monitored for 10 days, and body weights were recorded on days 0, 5, and 10. On day 10, tissue samples from liver and duodenum were collected and fixed in 10% formalin for histopathological analysis. Results showed that *G. duodenalis*-challenge significantly reduced the average body weights of mice on days 5 and 10 post-challenge. Histopathological results revealed that *G. duodenalis*-challenge caused hepatic blood vessel congestion, disappearance of hepatic cords, and pyknosis of hepatocytic nuclei. The intestinal histology was remarkably impacted with hyperplasia of goblet cells, cellular infiltration in the lamina propria, and extensive enterocyte damage. Additionally, numerous giardial parasites were observed in the mucus between the intestinal villi and lamina propria. These findings suggest that *Giardia duodenalis* isolates induce significant histopathological changes, primarily in the liver and intestines.

Keyword: *Giardia duodenalis*, Histopathology, Balb/c, Liver, intestine

INTRODUCTION

Giardia duodenalis, also known as *Giardia intestinalis* and *Giardia lamblia*, is a flagellated protozoan parasite that colonizes in the small intestine of various hosts, including humans and dogs. This organism is the causative agent of giardiasis, a gastrointestinal disease characterized by symptoms such as diarrhea, abdominal

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discomfort, and dehydration [17]. The transmission of *Giardia duodenalis* through contaminated water, food, or soil major concern globally due to its prevalence and public health implications [14]. Studies have increasingly focused on the zoonotic potential of *Giardia duodenalis*, exploring its transmission between animals and humans [1]. This investigation into both human and canine hosts provided critical insights into the genetic diversity of the parasite and potential cross-species transmission dynamics [13]. Dogs, being common domestic animals, may act as reservoirs for zoonotic strains of *Giardia duodenalis*, potentially impacting human health [5]. The detection of similar or identical strains in both human and canine hosts suggests the possibility of interspecies transmission, raising significant questions about the epidemiological role of pets in giardiasis [4]. To delve deeper into the pathogenicity and cross-species implications of *Giardia duodenalis*, the histopathological effects of dog-derived *Giardia* isolates on liver and intestinal tissues in a Balb/c mouse model. This approach simulates a zoonotic transmission scenario, providing a controlled environment to assess the impact of these isolates on mammalian hosts [11]. The outcomes of this study are expected to enhance our understanding of the zoonotic potential of *Giardia duodenalis*.

MATERIALS AND METHODS

Ethical statement and animals

The study was conducted based on the ethical approval received from the local Research Ethics Committee, College of Veterinary Medicine, University of Baghdad (Approval Number 2518 P.G. dated 12th November 2023).

Giardia duodenalis

Stool samples were collected from humans and fecal samples from dogs. To detect *Giardia* cysts, fecal flotation with centrifugation was performed for 3 to 5 minutes at a speed of 1,500 to 2,000 rpm. The infection dose was calculated to be 2×10^4 cysts per milliliter using a hemocytometer and diluted with normal saline [20].

Experimental study

A total of twenty healthy albino female mice of the BLAB/c strain (*Mus musculus*), with an average age of 8 weeks (± 2.4 days) and an average weight of 19.8 ± 0.5 g, were obtained and housed in the animal facility at the College of Veterinary Medicine, University of Baghdad. The animals underwent a 7-day acclimatization period, during which they were housed in plastic cages measuring 20×30×50 cm with stainless-steel wire mesh lids. They were provided commercial rodent feed pellets and tap water available *ad libitum*. Environmental conditions were maintained at 25 (± 5) °C and 50 (± 5)% relative humidity, following a 12-hour light and 14-hour dark cycle. Mice were randomly divided into two groups of ten each. The first group served as a control and orally received 0.3 mL of normal saline. The second challenged group received *G. duodenalis* via oral administration at a single dose of 2×10^4 cyst/mL. The dose of infection was calculated using a Hemocytometer diluted with normal saline. Clinical signs were monitored and recorded daily for 10 days. Body weight measurement was performed on days 0 (before the challenge), 5, and 10 (after the challenge) [20]. At day 10, the animals were sacrificed for macroscopic pathological changes and histopathological examination [16].

Postmortem examination

On day 10 post-infection, animals from both groups were scarified using an overdose of chloroform anesthesia. Postmortem examination was performed to evaluate the macroscopic pathological changes focusing on the liver, spleen, and intestines. This timeframe was chosen based on the expected progression of giardiasis symptoms and to capture the peak of pathological changes induced by the parasite.

Histological examination

Following postmortem examination on day 10 post-infection, liver and duodenum samples were harvested from two animals/each group and preserved in 10% buffered formalin for two days. Samples were then sectioned to 0.5 cm thickness and placed in plastic cassettes [6].

RESULTS AND DISCUSSION

Clinical Signs and Body Weight

The experimental group, challenged with *G. duodenalis*, showed symptoms indicative of infection, including lethargy and abdominal distension. Beginning on day 5 post-challenge, symptoms such as watery diarrhea, dehydration, and behavioral depression were observed. The clinical signs observed in *G. duodenalis*-infected mice, including lethargy, diarrhea, and wasting symptoms beginning around day 5 post-infection, are consistent with giardiasis pathogenesis in humans and animal models. The abdominal distension and watery, yellow diarrhea likely result from intestinal villi damage and impaired nutrient absorption. The significantly lower body weight of infected mice compared to controls correlates with the histopathological changes seen in the small intestine. Villous atrophy and enterocyte loss lead to reduced surface area for nutrient absorption, which could contribute to malabsorption, diarrhea, and delayed growth seen in giardiasis. Indeed, studies have demonstrated associations between increased intestinal injury scores and decreased weight gain in *Giardia* infection [18]. The extensive mucosal damage, combined with diarrhea-associated dehydration, is a probable mechanism underlying the observed suppression of weight gain. The additional histological changes observed in the liver may also play a role in the wasting symptoms. Hepatic inflammation can lead to impaired synthesis of proteins and nutrients critical for growth. Analysis of serum liver enzymes and metabolic markers could provide more insight into the systemic effects of liver damage during *Giardia* infection. The significant weight loss appears to be a consequence of the profound structural and functional disruption of the intestines and liver [12].

Postmortem examination

After the 10-day period of infection with *G. duodenalis* was over, a postmortem examination was conducted. In the liver, there was notable hepatomegaly, with the liver presenting an enlarged appearance and a darkened, reddish-brown color, which could indicate congestion or potential hepatic inflammation. The liver's surface appeared smooth, and its increased size. Clinical signs of abdominal distension were observed prior to euthanasia (Figure 1). The spleen exhibited signs of splenomegaly. It was visibly enlarged and darker in color, indicating possible hyperactivity or an immune response to the

infection. The splenic enlargement provided a macroscopic correlation to the systemic involvement suggested by pre-euthanasia observations of lethargy and potential abdominal discomfort. The intestines were markedly distended and filled with watery ingesta, indicating severe gastrointestinal involvement. This distension likely resulted from inflammation and fluid accumulation, reflecting the diarrhea and dehydration clinically observed during the course of the infection. These findings align with the known pathophysiological effects of *Giardia duodenalis* infection and provide macroscopic evidence of its impact on the gastrointestinal system and associated organs [3].



Figure 1: Post mortem appearance congestion of liver and spleen and intestinal swelling filled with watery ingesta after 10 days of oral infection with *G. Duodenalis*

The observation of hepatitis and splenomegaly during the postmortem examination underscores the systemic nature of *Giardia duodenalis* infection. These findings suggest that the impact of the parasite extends beyond the gastrointestinal tract, affecting other organs such as the liver and spleen. The liver changes are particularly significant, as they indicate a possible direct or indirect effect of the parasite or the host's immune response to the infection [8]. Similarly, the splenic enlargement hints at a heightened immune system activity, possibly in response to the ongoing infection. These systemic effects highlight the complexity of *Giardia duodenalis* pathogenesis and its potential to cause widespread physiological disturbances in the host [3]. The observed congestion in the liver and spleen can be indicative of a systemic inflammatory response to the *Giardia duodenalis* infection. This congestion might be reflective of increased blood flow due to inflammatory processes or a direct impact of the parasite on these organs. This aligns with our histopathological findings of liver tissue alterations and suggests a broader systemic involvement than just gastrointestinal symptoms. The marked swelling of the intestines filled with watery ingesta is a direct manifestation of the gastrointestinal impact of *Giardia duodenalis*. This observation corroborates our histological findings of intestinal damage, including villous atrophy and enterocyte disruption. The swelling can be attributed to inflammatory edema

and disruption of normal fluid absorption in the intestines, leading to watery diarrhea, a hallmark clinical symptom of giardiasis. The postmortem findings provide a macroscopic perspective that complements our microscopic observations. The severity of the intestinal swelling and liver congestion observed correlates with the clinical signs of lethargy, abdominal distension, and watery diarrhea noted in the infected mice. This correlation underscores the link between the observed histopathological changes and the clinical manifestation of the disease [9].

Histopathological Findings

Control Group (Normal Saline Dosage)

Histopathological examination of liver sections from control group revealed normal hepatocyte and sinusoid structure (**Figure 2**). The intestinal sections showed typical epithelium of villi, lamina propria, and Brunner's glands (**Figure 3**).

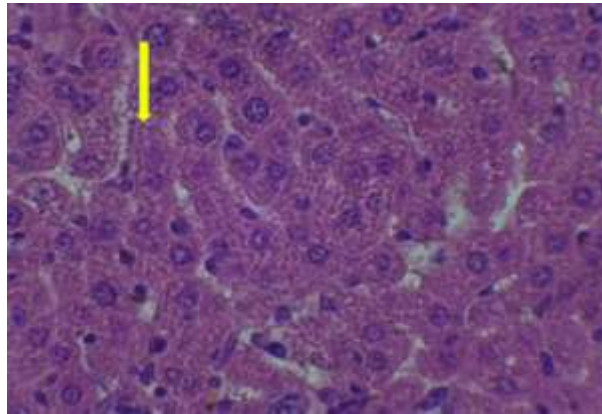


Figure 2. Histopathological section of liver female Balb/c mice from control group which given normal saline shows hepatocytes, and sinusoids (H&E, 40×).

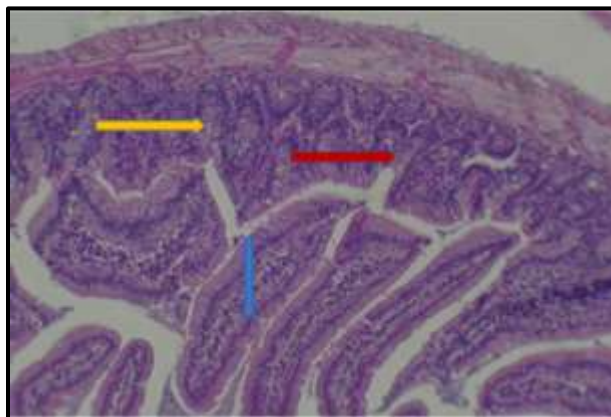


Figure 3. Histopathological section of the duodenum of female Balb/c mice from the control group showing normal appearance of the epithelium of the villus (blue arrow), lamina propria (red arrow), and Brunner's glands (yellow arrow) (H&E stain, 100×).

Group 2 (infected with *Giardia duodenalis*)

In contrast, liver sections from *G. duodenalis*-infected mice showed pathological changes including congestion of blood vessels, pyknosis, cytoplasmic granulation, and binucleated hepatocytes. Aggregations of macrophages surrounding congested blood vessels and damaged hepatocytes were also observed (Figure 4).

Intestinal sections from infected mice displayed goblet cell hyperplasia (Figure 5), increased cellularity of the lamina propria, and extensive damage to enterocytes with numerous *Giardia* trophozoites and cysts present between villi (Figures 6,7). Additional intestinal abnormalities included enterocyte loss, villous atrophy, vascular dilation, and lymphocytic infiltration.

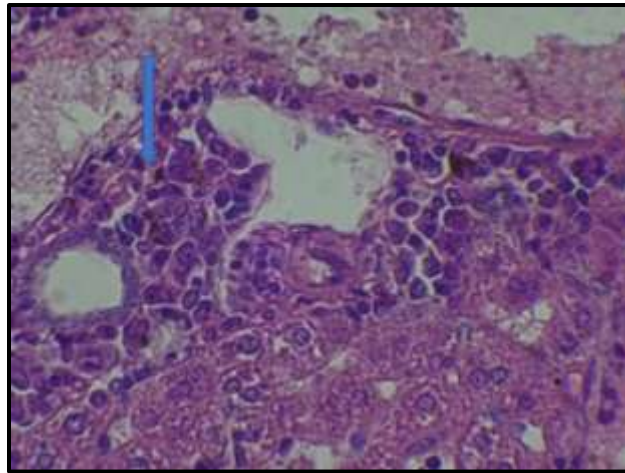


Figure 4. Histopathological section of the liver of group 2 infected with *Giardia duodenalis* shows aggregation of macrophages around congested blood vessels, pyknosis, and binucleated hepatocytes (H&EX400).

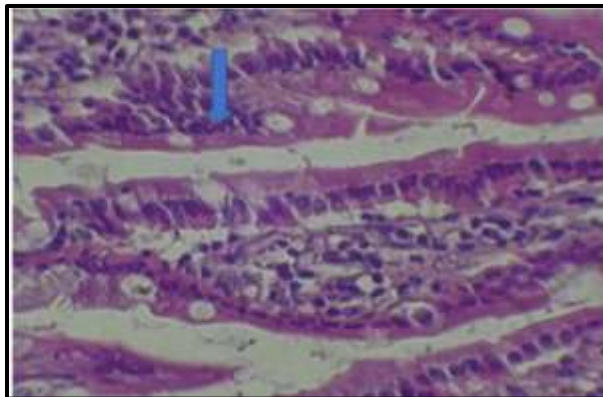


Figure 5: Histopathological section of the intestine of group 2 infected with *Giardia duodenalis* shows hyperplasia of goblet cells and cellular lamina propria. (H&EX400).

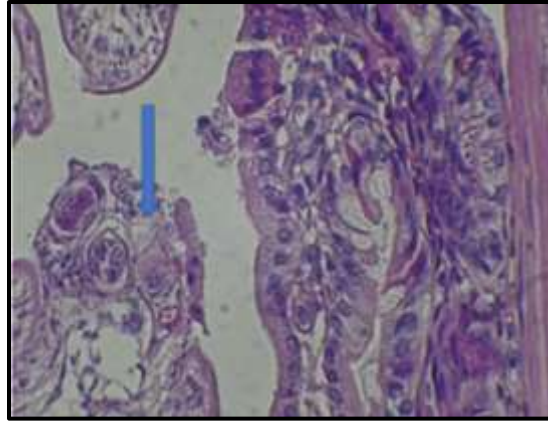


Figure 6. Histopathological section of the intestine of group 2 infected with *Giardia duodenalis* shows to enterocytes damage & villi atrophy, dilated blood vessels, different stages of trophozoites and cysts (H&EX400)

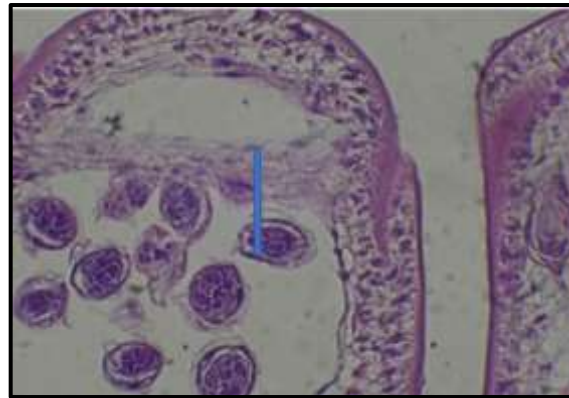


Figure 7: Histopathological section of intestine of group 2 infected with *Giardia duodenalis* shows physical barriers between the enterocytes with different stages of trophozoites and cysts, enterocytes damage (H&EX400)

The histopathological changes observed in the liver and intestine of *G. duodenalis*-infected mice are consistent with previous reports. The normal hepatic and intestinal morphology seen in control mice has been previously described [15]. Also the pathological changes were observed grossly and histopathological in intestine agreement with [19] revealed intestine changes including hyperplasia in colonic glands and mucosa, increase no. of goblet cells and aggregation of parasites in mucosa when infected *G. duodenalis*. The intestinal goblet cells and increased cellularity of the lamina propria are known consequences of giardiasis that aid mucosal immune defenses against the parasite [19]. Our findings of villous atrophy, enterocyte damage, and infiltration of *Giardia* forms have also been reported and likely result from direct contact-dependent effects of *Giardia* adhesion and toxin secretion [7]. As in Samra *et al.* study [21] the presence of *Giardia* in the liver suggests migration of the parasite from the intestines via penetrating of intestinal barriers, biliary transmission, or the portal circulation [22]. Unlike other intestinal pathogens associated with constrained linear growth that cause intestinal or systemic inflammation or both, *Giardia* seldom, *Giardia* results in linear growth deficits and gut permeability that

are dose-dependent and independent of intestinal markers of inflammation [12]. Adam [3] showed that understanding the parasite/epithelial cell crosstalk at the mucosal surfaces of the small intestine during giardiasis may provide novel insights into the mechanisms underlying the parasite-induced immunopathology and epithelial tissue damage, leading to malnutrition. Efforts to identify new targets for intervening in the development of intestinal immunopathology and the progression to malnutrition are critical. Morphological changes of hepatic *Giardia* may represent biological adaptation to the liver environment [10]. The liver damage induced by *G. duodenalis* infection, indicated by congestion, cellular degeneration, necrosis, and inflammation, aligns with earlier studies [2].

Conclusion

This study provides evidence of the significant pathophysiological impact of *Giardia duodenalis* infection in a Balb/c mouse model. The clinical signs observed, including lethargy, abdominal distension, and watery diarrhea, were corroborated by the postmortem findings of liver congestion, splenomegaly, and intestinal swelling with watery ingesta. These observations confirm the systemic nature of the infection, not limited to gastrointestinal disturbances but also affecting other organs, such as the liver and spleen, indicative of a broader immune response. Histopathological examination further substantiated these findings, revealing cellular and tissue-level changes within the liver and intestines that aligned with the clinical and macroscopic postmortem observations. The presence of binucleated hepatocytes, congestion, and hyperplasia of goblet cells in the intestines provided a microscopic view of the infection's impact. The similarity of clinical signs with postmortem and histopathological findings highlights the robustness of the Balb/c mouse model in mimicking the disease's progression and pathogenicity.

These results contribute to our understanding of *Giardia duodenalis* as a zoonotic parasite with significant implications for both veterinary and human medicine. Future studies should aim to further elucidate the molecular mechanisms underlying the observed pathological changes and to explore potential therapeutic interventions. Public health strategies must also consider the zoonotic potential of *Giardia duodenalis*, especially in contexts where humans and domestic animals closely interact.

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التأثير النسيجي المرضي لعزلات الجيارديا الاثني عشرية على أنسجة الكبد والأمعاء في نموذج فئران Balb/c

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الملخص

هذه الدراسة، قمنا بتوسيع هذا العمل من خلال فحص التأثيرات النسيجية المرضية لعزلات طفيلي الجيارديا المعزولة من الانسان والكلاب باستخدام نموذج الفأر/BALB متوسط العمر: 8 أسابيع ، n=20 تم تقسيم الفئران 2 ± 4 يوم؛ والوزن: 5.0 ± 8.19 جم) بشكل عشوائي إلى مجموعتين من عشرة.

تلقت مجموعة السيطرة التي لم يتم تحديها 3.0 مل من المحلول الملحي الطبيعي عن طريق الفم، في حين تلقت المجموعة التي تم تحديها بكتيريا الاثني عشر بكتيريا الاثني عشر بمعدل 2×10^4 كيسة/مل في 3.0 مل عن طريق الفم. تمت مراقبة العلامات السريرية لمدة 10 أيام، وتم تسجيل أوزان الجسم في الأيام 0 و 5 و 10. في اليوم 10، تم حصاد عينات الأنسجة من الكبد الأيمن والاثني عشر وتثبيتها في 10٪ من قتل بشكل كبير من متوسط *Giardia duodenalis* الفورمالين لتحليلها النسيجي.

أظهرت النتائج أن تحدي أوزان الجسم لدى الفئران في اليومين 5 و 10 بعد التحدي. أظهرت النتائج التشريحية المرضية أن تحدي يسبب احتقان الأوعية الدموية الكبدية واختفاء الحبال الكبدية وتخلل نوى الخلايا G. Duodenalis الكبدية. تأثرت الأنسجة المعوية بشكل ملحوظ بتضخم الخلايا الكأسية، والتسلل الخلوي في الصفيحة المخصوصة، وتلف الخلايا المعوية بشكل كبير. بالإضافة إلى ذلك، لوحظ وجود العديد من طفيليات الجيارديا في المخاط الموجود بين الزغابات المعوية والصفيحة المخصوصة. تشير هذه النتائج إلى أن عزلات الجيارديا الاثني عشرية تحدث تغيرات نسيجية مرضية كبيرة، خاصة في الكبد والأمعاء

الكلمات الدالة: الجيارديا الاثني عشرية، علم الأمراض النسيجي، Balb/c، الكبد، الأمعاء

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